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THE VASCULARIZATION OF THE PERIPHERAL NERVE OF CHICKEN AND RAT

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Abstract

The vascular system of the sciatic nerve of chicken and rats was examined by means of the microcorrosion casting technique and freeze-broken specimens.

The main epineural vessels form two lateral and interfascicular vascular bundles which anastomose with one another and also with the peri- and endoneural plexuses. On epi-and perineural vessels one can find morphological correlates for regulative means such as sphincters. Even the endoneural vessels depict numerous anastomoses.

The proximity of the vessels as well as the great number of anastomoses suggests a considerable compensatory potential with an adaptable perfusion rate in case of a partial breakdown of a plexus.

Key Words: peripheral nerve, sciatic nerve, blood supply, vascularization, corrosion cast, chicken, rat, scanning electron microscopy.

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Introduction

Fetterman and Spitler (1940) -among other authors- concluded that vascular diseases lead to ischemic neuropathies. Basing his conclusions on the first systematic studies Adams (1942) distinguished three kinds of vessels: nutritive vessels, epineural vessels, and an intrafascicular plexus. Blunt (1959), Waksman (1961), and Lang (1962), confirmed these results.

A very important contribution came from Lundborg and Branemark (1968). They discovered that the microcirculation consisted of two integral systems independent from one another: the "extrinsic" consisting of nutritive epineural vessels and a perineural plexus, and the "intrinsic" consisting of an intrafascicular plexus. According to these authors the intrinsic system is able to undertake the nutrition of the whole nerve if the extrinsic system breaks down, e.g., through a surgical mobilization of a larger part of the nerve. Hiramatsu (1982), who has carried out the only work on the microvasculature of peripheral nerves using corrosion casting techniques, partially agreed with these findings.

The aim of this study is to demonstrate the vascularization of the peripheral nerve by means of microcorrosion casting and freeze broken nerves in order to give an improved three-dimensional illustration and to get basic knowledge for further experimental studies.

Materials and Methods

For our studies 14 female, 18-week-old chickens of the HNL-breed with a weight between 735-1135 g were obtained. Additionally, the sciatic nerves of 11 12-week-old Wistar rats of both sexes with a weight between 160-240 g were studied.

Corrosion casting technique:

Following recommendations of Lametschwandtner et al. (1984) and Hodde et al. (1980) we have chosen the following procedure: In deep pentobarbital anaesthesia (Nembutal (R), 80 mg/kg body weight intraperitoneally) the animals were thoracotomized 30 minutes after intraperitoneal application of heparin (Liquemin (R), 5000 IU/kg body weight). The rats were exsanguinated with up to 250 ml 0.9% NaCl-solution, the chicken with up to 450 ml 0.7% NaCl-solution. The solution was ap-

plied by means of an olive-tipped cannula inserted into the left ventricle. The perfusion pressures were between 80-110 mm Hg, the solution temperatures 41°C for chickens and 37-39°C for rats. Then the animals were fixed for a maximum of 5 minutes in Karnovsky's solution (1965) (pH 7.40, 1660 mosmol) to a total of 450 ml. In the region of the lower thoracic vertebral bodies, the thoracic aorta was dissected, the button-cannula inserted and advanced into the right and left common iliac arteries, respectively, and the external iliac arteries. In these vessels 20 ml (rats) or 50 ml (chickens) of the casting medium Mercox CL-2B or Mercox CL-2R (Japan Vilene Comp. Ltd., Japan) mixed with 1.75 % catalyzing substance were injected. After 4 h in a water-bath at 40°C the specimens were completely hardened. Then the sciatic nerve was dissected and macerated 12-18 h with a quarternary ammonium base (Soluene 350,Packard). After drying and mounting on holders the speci-mens were sputtered with gold in an argon-atmos-phere and examined with a Stereoscan 180 scanning electron microscope (Cambridge) at 10-13 kV acce-leration voltage.

Freeze-broken specimens:

After perfusion with saline and fixation -as described above- parts of the nerves of approximately 10 mm length were dissected free and post-fixed for 4 h in Karnovsky´s solution (1965) at 4°C. The specimens were next washed in cacodylate-buffer and contrasted in osmic-acid-solution (Dalton, 1955). In ascending grades of alcohol and amyl acetate they were dehydrated. From the pure amyl acetate the specimens were transferred to fluid propane gas in a liquid nitrogen environment. After complete freezing they were broken with a scalpel. Then the specimens were retransferred to amyl acetate, dried in liquid CO_2 by the critical point method, mounted on specimen holders, and sputtered in an argon-atmosphere. They were examined with a Stereoscan 180 scanning electron microscope (Cambridge) at an acceleration voltage of 15-20 kV.

Results

The sciatic nerve of the rat as well as that of the chicken not only receives arterial branches mainly from the external sciatic artery but also from the smaller arteries of the neighbouring tissues, e.g., muscles. These nutritive vessels have a connective tissue of their own, the mesoneurium (cf. Smith, 1966; Nobel and Black, 1974). They flow into the epineural vessels.

The epineural vessels

The main epineural vessels form a lateral vascular bundle on both sides of the sciatic nerve and an interfascicular vascular bundle along the axial direction of the nerve (fig. 1). The main arterial and venous epineural vessels are connected by transverse (fig. 1) or diagonal anastomoses (fig. 2). A plexus-like vascular network is formed on some main epineural vessels and several small vessels and capillaries. One artery is usually accompanied by one or two parallel running veins (fig. 2). This arrangement is discontinued when the larger muscular branches of the nerve ramify. These do not further have the arrangement of the epineural vessels as typical in the sciatic nerve (fig. 3).

Arteries as well as veins show a circular contractive profile probably having a regulative function (fig.4). The endothelia of the main arteries have an ovoid shape and are orientated in a longitudinal direction (fig. 4 inset). The endothelia of the main veins are not ovoid but irregular.

The perineural vessels

The perineural as well as the epineural vessels are characterized by their longitudinal arrangement. However, the partly wave-like vessels have several cross-connections to one another (fig. 5). The vascular density of this plexus seems to be more intense in the chicken than in the rat. In the border region of the peri- and epineural plexuses numerous anastomoses can be seen which are characterized by sphincter-like constrictions (fig. 6). In vessels running inwards and outwards from the peri- to the endoneural vessels, we detected a considerable amount of constrictions and dilatations (fig. 7 a and b). We have observed such changes in the diameters both in arteries and veins. Of course it was not always possible to define the border between peri- and endoneural vessels in the corrosion casts with certainty. This pertained mainly to the veins.

The endoneural vessels

The vessels of the endoneural region of the fascicles mainly have an axial arrangement, too. They depict a wave-like form as the perineural vessels (fig. 8). There are not as many circular constrictions as in the peri- or epineural vessels. Between the vessels there are several diagonally and longitudinally orientated anastomoses (fig. 9).

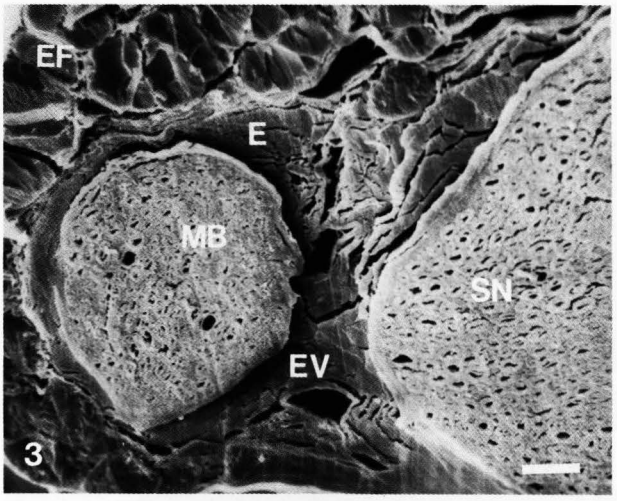
In freeze-broken specimens and corrosion casts we could not find any significant differences between the densities of endoneural vessels of the rat and those of the chicken.

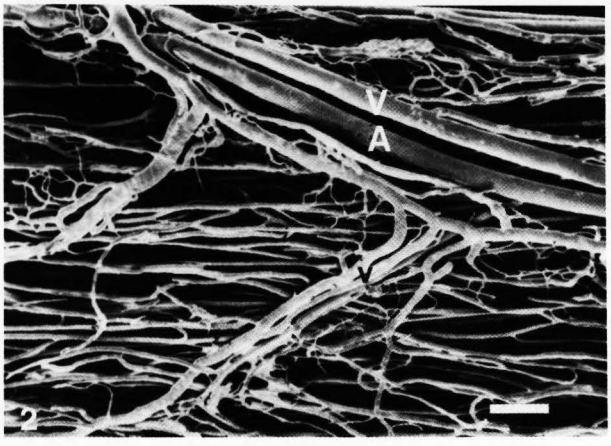
Fig. 1: Vascular corrosion cast of the sciatic nerve (chicken). Demonstration of epineural vessels. I = interfascicular, L = main lateral epineural vessels, V = venous anastomoses between main vessels. Bar = 300 µm

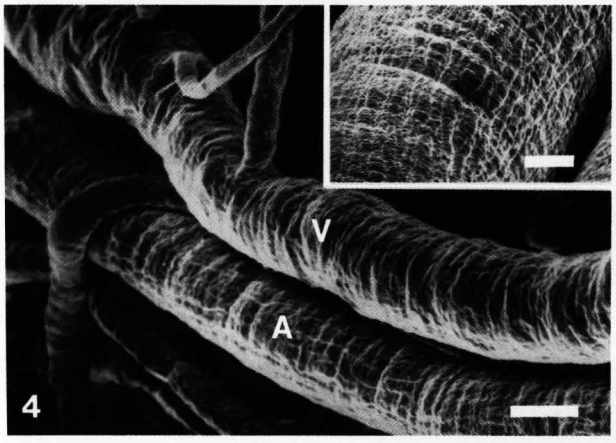
Fig. 2: Vascular corrosion cast of the sciatic nerve (chicken). v = venous anastomosis of main epineural vessels; A = artery, V = vein. Bar = 300 µm

Fig. 3: Freeze-broken specimen of sciatic nerve (rat) in the region of branching muscular ramus. E = epineurium, EV = epineural vessels, EF = epineural fat, SN = sciatic nerve, MB = muscular branch. Bar = 30 µm

Fig. 4: Corrosion cast of chicken sciatic nerve. Surface structure of epineural arteries (A) and veins (V). Note the circular contraction profiles. Inset: longitudinally orientated endothelial cell-borders of an artery. Bar = 20 µm. Bar inset = 10 µm







Discussion

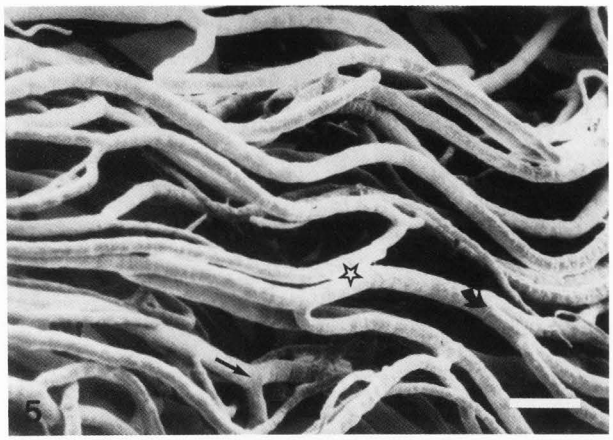
There are already some detailed studies about the vascularization of the peripheral nerve of animals (e.g., rabbits, Lundborg and Branemark, 1968) as well as of human beings (e.g., Lundborg, 1979). These studies still remain an important model for the microcirculation of peripheral nerve. But it is not yet possible to give a correct view of the vascularization with intravital microscopic or microangiographic methods.

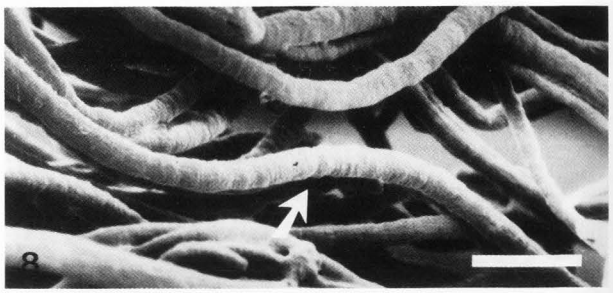
Stöhr (1980), Carter et al. (1972), Eames and Lange (1967), and Raff and Asbury (1968) regarded a disturbed microcirculation as a cause for the development of many neuropathies. In order to verify damaging influences such as trauma, pressure, vibration, or metabolic imbalance, to the nerve or its vessels with a model it was necessary to have an in toto demonstration of the vascularization.

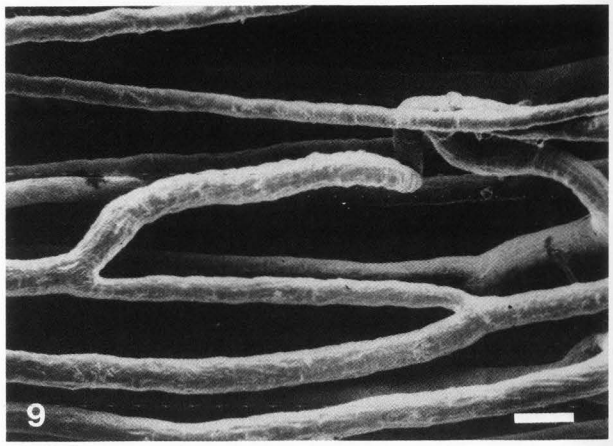
This is possible by corrosion casting: Hiramatsu (1982) demonstrated the microvasculature of peripheral nerves in dog and human samples with this technique. Together with him we widely confirm the circulatory model established by Lundborg and Branemark (1968) and subsequently improved by Lundborg (1975).

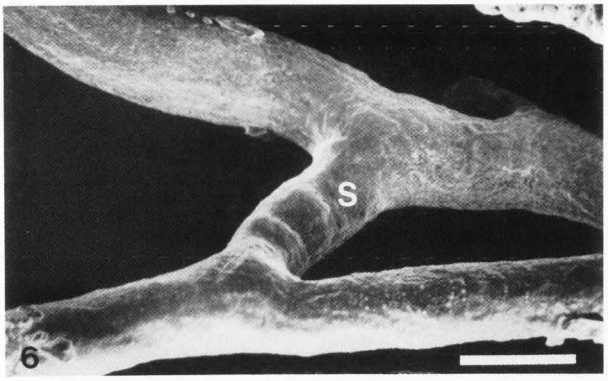
In addition we discovered the presence of sphincter-like structures by scanning electron microscopy in the vessels of all plexuses, though less frequent in the endoneural plexus. The circular constriction profiles are probably caused by smooth muscle constrictions of the vascular wall probably leading to intravital changes in the diameter and bloodflow. Although these constrictions are also visible in some micrographs, Hiramatsu (1982) did not comment on them. The great number of cross-communications between epi-, peri- and endoneural vessels might explain why a short breakdown of one of those systems (e.g., as a consequence of a mobilization of a nerve) does not lead to a generalized ischemia of the whole nerve. The high vascular density might confirm Lundborg´s findings (1975) that only a part of the whole endoneural plexus is perfused and that there are vessels in reserve which are brought into play when required. It is also possible that the intense proximity of the vessels in the epineural plexus supports the stabilization of the nerve in its surroundings.

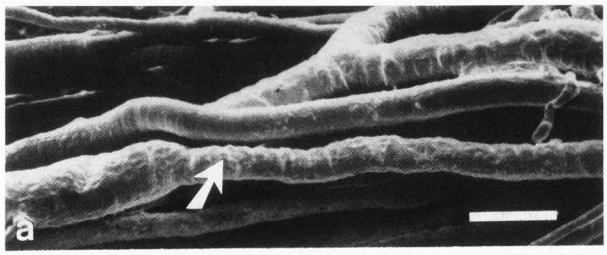
The meandering of the endoneural capillaries is probably responsible for the fact that the











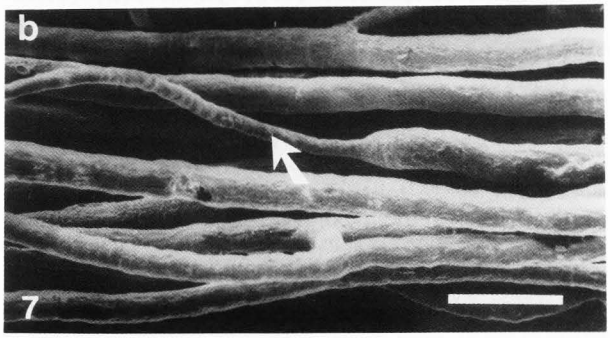


Fig. 5: Survey of perineural vessels of the sciatic nerve (chicken). Note the side to side anastomosis (*) and the dichotomous branching (➧). Bar = 100 µm

Fig. 6: Shunt connection (S) of two arterioles between peri- and endoneural plexus with sphincter-like impressions (Corrosion cast, rat). Bar = 30 µm

Fig. 7 a, b: Long constrictions of venous vessels (➧) in the border region of peri- and endoneural plexus (Corrosion cast, chicken). Bar = 100 µm

Fig. 8: Corrosion cast of chicken sciatic nerve. Endoneural capillaries with sphincter-like constriction of lumen (➧). Bar = 50 µm

Fig. 9: Anastomoses between endoneural vessels of chicken sciatic nerve. Vascular corrosion cast. Bar = 50 µm

nerve can be distended to 115 % of its length without any nutritive harm (Lundborg and Rydevik, 1973). Waksman (1961) and Lang (1962) stated that in the endoneural space mainly capillaries are to be found. Together with Hiramatsu (1982) we can not confirm this finding.

Altogether, our studies of the vascularization of the sciatic nerve showed that there are no significant differences between rats and chickens so that experimental results in both species can then be compared with one another.

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Discussion with Reviewers

E.D.F. Motti: Why did you perfuse the wash-out solution through the heart and inject the casting medium through the branches of the descending aorta?

Authors: By the rapid access to the vascular system via the heart for rinsing and fixing coagulation should be avoided. The casting medium was injected into branches of the abdominal aorta to reduce the required volumes.

F. N. Low: In figure 2 the vessels labeled as veins seem to be continuous with those closely resembling those labeled arteries. What criteria are used to identify the two vascular types?

Authors: Besides the branching mode the most important criterion for the identification was the form of endothelial imprints (Miodonski A, Hodde KC, Bakker C (1976) Rasterelektronenmikroskopie von Plastik-Korrosions-Präparaten: morphologische Unterschiede zwischen Arterien und Venen (Scanning electron microscopy of corrosion casts: morphologic differences between arteries and veins). BEDO 9, 435-442.).

F. N. Low: In figure 4 why does the artery show endothelial margins so much more clearly than the vein? Is this characteristic of these preparations?

Authors: This is not characteristic but should be regarded as an artifact. We suppose that the intravascular pressures decrease due to numerous constrictions and dilatations so much that the filling and replication quality especially of the veins suffers.

E.D.F. Motti: How do you explain the obvious lack in your illustrations of a capillary bed stretching from arteries to veins? In case you think the capillaries were actually injected with the casting compound, which precautions did you observe to prevent damage to the finest vascular ramifications in the casted sciatic nerve during dissection preliminary to corrosion?

Authors: It might be possible that the numerous constrictions and sphincter-like structures have promoted an incomplete filling of the capillary bed. All specimens were dissected with razor blades under a stereo loupe to avoid damage to the injected vessels. Sometimes we have had problems during maceration: Independent from the concentration of the maceration solution (Soluene) we have observed several times distinct swelling of the nervous tissue, which might lead to damages, too.

E.D.F. Motti: The interesting feature of "circular constrictions", that you recognize both in arterial and venous casts, was it observed in all of your preparations? You report the mentioned constrictions to predominate in arterio-arterial

anastomoses (Fig. 6). I noted a similar phenomenon in the pial vessels of the rat (Motti EDF, Imhof HG, Martinez Garza J, Yasargil GM (1987); Vasospastic phenomena on the luminal replica of rat brain vessels. Scanning Microsc. 1, 207-222.) and advanced an interpretation based on the synergic influences of a peculiar experimental setting (causing high injection pressure) and the physiological property of localized sensitivity to heightened intravascular pressure gradients. Do you think similar factors could be at work in your experiment as well?

Authors: The described constrictions were observed as a constant pattern. We regard your interpretation as conclusive, although we have seen this phenomenon in animals injected with lower pressures as usual, too. Further transmission electron microscopic and histochemical studies should clarify as to whether these vessels differ in their ultrastructure from other vessels.